**Abstract**

Cytochrome $b_5$ is an electron transport protein of a clinically prominent mixed-function oxygenase (MFO) system. This system participates in the first stage of xenobiotic biotransformation. The hydrofility of xenobiotics is increased by introduction of an oxygen atom into their structure. The MFO system also activates or deactivates certain drugs and carcinogens. Cytochrome $b_5$ affects reactions catalyzed by the terminal oxygenases of the system - cytochromes P450. Electrons are donated to cytochrome $b_5$ by redox partners NADH:cytochrome $b_5$ reductase and NADPH:cytochrome P450 reductase.

The aim of this work was to demonstrate capability of photo-crosslinking approach to fixate transient interactions within MFO system. Covalent complexes obtained by this technique could be further analyzed by mass spectrometry, which can provide structural information about the binding sites of the proteins.

We prepared a mutant cytochrome $b_5$ comprising photo-reactive methionine analogue in the position 41 of the sequence. We expressed the protein employing *E. coli* B834 (DE3) auxotrophic strain in 300 ml of the limit medium supplemented with L-2-amino-5,5-azi-hexanoic acid (photo-methionine). The rate of the unnatural amino acid incorporation was determined by mass spectrometry and it was about 40 % after 16 hours of protein expression. We isolated 15.4 nmol of mutant cytochrome $b_5$ in total and its concentration was 76.8 μmol/l.

The photo-reactive cytochrome $b_5$ M41 with with individual redox partners was reconstituted with liposomes and activated by long-wave UV radiation.

We successfully formed cytochrome $b_5$ – cytochrome P450 covalent complexes and thus showed photo-crosslinking capability of the isolated protein. Unfortunately no covalent complexes with NADH:cytochrome $b_5$ and NADPH:cytochrome P450 reductases were obtained by this technique.

**Keywords:** cytochrome $b_5$, photoreactive amino acids, protein cross-linking, protein-protein interactions, cytochrome P450, NADH:cytochrome $b_5$ reductase, NADPH:cytochrome P450 reductase.

In Czech